

EXTENDED REPORT

Tissue crossmatch before corneal transplantation

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Aim: To investigate the influence of anticorneal antibodies on the outcome of transplantation in recipients before penetrating keratoplasty.

Methods: 100 consecutive penetrating keratoplasties performed between July 1995 and June 1996 were included in this study. Donor corneoscleral rim cryosections were incubated with recipient serum. Fixation of recipient IgM on donor corneas was revealed with a polyclonal anti-human μ -chain antibody. The mean follow up time was 61 months. Five years after transplantation, 63% of patients were available for follow up. During the follow up period, 15% of keratoplasties failed because of irreversible rejection.

Results: Anti-donor stromal lamellae IgM were found in 14 recipients. Anti-donor keratocyte IgM were present in 28 recipients. Dilution of recipient serum (up to 1/800) did not modify the results of positive crossmatch assays. Crossmatch assays performed with positive recipient sera and negative donor corneoscleral rims were negative. No significant influence of IgM crossmatch results on graft survival and rejection-free graft survival was found.

Conclusion: Donor specific anticorneal stroma IgM were found in 28% of recipients before transplantation. This pre-immunisation was not associated with a higher risk of transplantation rejection.

Testing recipient serum before transplantation for the presence of antibodies directed against donor antigens is a relevant method for preventing acute rejection of organ transplants. In the field of corneal transplantation, pretransplant antibodies directed against donor human leucocyte antigens were shown not to increase the risk of rejection in several studies. In these studies, crossmatching was performed using the microlymphocytotoxicity method. Boisjoly *et al*¹ reported that donor-recipient crossmatching, which was performed in 342 grafts with 20 positive crossmatch assays (5.8%) and 322 negative ones (94.2%), was not a significant risk factor for endothelial allograft reaction. The Collaborative Corneal Transplantation Studies group² compared 18 high risk recipients with a positive crossmatch to 19 high risk recipients with a negative crossmatch and found no significant differences between both groups for the 2 year failure rate and irreversible rejection rate. Des Marchais *et al*³ also reported no significant influence of a positive crossmatch on the 3 year graft rejection rate in 759 consecutive corneal transplantations (61 patients with a positive crossmatch and 698 with a negative one) but found an increased risk of rejection associated with a positive crossmatch in recipients with a rejection history or with a previous graft. Interestingly, post-transplant antibodies directed against donor human leucocyte antigens were shown to be associated with a decrease in the 3 year rejection-free graft survival.⁴

The rationale for this crossmatch is that corneal allograft rejection could be directed against donor human leucocyte antigens (HLA). However, non-HLA antigens also have a recognised role in corneal allograft rejection. It has been shown for ABO incompatibility and for Lewis incompatibilities.²⁻⁵⁻⁸ Thus, it is of interest to investigate the influence of pretransplant alloantibodies directed against non-HLA antigens (that is, minor histocompatibility antigens) on graft rejection. Assuming that minor histocompatibility antigens could have a significant role in corneal graft rejection, they should be expressed by corneal cells. In this study we investigated the influence of pretransplant antibodies directed against donor corneal antigens on postoperative graft rejection using a tissue crossmatch method.

METHODS

Patients

This study was carried out according to the tenets of the Declaration of Helsinki. It was approved by an ethics committee and each patient gave informed consent. It included 100 consecutive penetrating keratoplasties (100 recipients) performed between 1995 and 1996.

All transplants were performed at a single institution by two surgeons (VMB and LL).⁹ All donor buttons were punched from the posterior corneal surface by using the Hanna device. The mean donor trephination size was 8.17 (SD 0.24) mm. The mean recipient trephination size was 7.92 (0.23) mm. All patients were treated with topical dexamethasone (1 mg/ml) and then neomycin (3400 IU/ml). This treatment was tapered over a period of several months, from four times daily postoperatively to once daily at 6 months, without standardisation of postoperative steroid management. Most patients remained on a dosage of one drop every 2 days from 9 months after surgery until the time of suture removal. Sutures were removed routinely between 12 and 18 months. Patients who developed signs of transplant rejection were treated with a variable dosage and route of steroids ranging from topical dexamethasone every waking hour during the first 7 days to a subconjunctival dexamethasone (2 mg) injection once a day or systemic methylprednisolone (500 mg intravenously once a day for 3 days).

The mean age of the patients was 57 (SD 22) years. Patient characteristics are shown in table 1. High risk recipients were defined as having a vascularised cornea (two or more quadrants of corneal vascularisation) or a history of irreversible corneal allograft rejection.

Patients were hospitalised up to graft re-epithelialisation. They were then examined at 2 weeks, 1, 3, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72, and 84 months after surgery. Patients who lived outside France were examined by the ophthalmologist who referred them to us. The mean follow up time (table 2) was 61 (SD 25) months. The criteria for graft failure were irreversible graft stromal oedema or corneal opacification. We defined rejection as graft failure with rejection line, graft infiltrates, keratic precipitates, graft vascularisation, ciliary

Table 1 Patient characteristics

Preoperative diagnosis	
Fuchs' dystrophy and ICE syndrome	6
Keratoconus	19
Bullous keratopathy	50
Other corneal dystrophies	5
Corneal scar, corneal ulcer, interstitial keratitis	9
Trauma	4
Regraft	7
Total	100
Recipient rejection status	
Low risk	78
High risk	22
Total	100

injection, or aqueous cells. The diagnosis of rejection was made only if the transplant had remained clear for an interval of at least two weeks after surgery.

Tissue crossmatch assays

Crossmatch assays

At the time of transplantation, the donor corneoscleral rim was placed in a sterile container with 5 ml of preservation medium. It was sent to the cornea bank at room temperature and then frozen in isopentane cooled in liquid nitrogen. Cryosections of 7 µm were prepared and were stored at -80°C before staining. After warming at room temperature, donor corneoscleral rim cryosections were incubated with 150 µl of preoperative recipient serum for 45 minutes at room temperature and then washed in phosphate buffered solution (PBS, pH 7.2) for 5 minutes three times. The following antibodies were used: peroxidase conjugated polyclonal anti γ-chain of human IgG and polyclonal anti µ-chain of human IgM (Sanofi Diagnostic Pasteur, Aulnay-sous-Bois, France). Cryosections were incubated with a solution of the polyclonal antibody in PBS for 45 minutes at room temperature. After washing in PBS, antibody binding was visualised by incubating the specimens in a Vector VIP SK-4600 solution (Vector Lab Inc, Burlingame, CA, USA) for 10 minutes. The specimens were then dehydrated, fixed, mounted, and observed under a light microscope. Optimal dilution of the primary anti-human IgG and IgM antibodies was first studied using cryosections of human lymph nodes and cryosections of human donor cornea, limbus, and trabeculum.

Donor controls

Cryosections of each donor corneoscleral rim were studied for the presence of donor IgG and IgM using the same procedure omitting the incubation of donor corneoscleral rim with recipient serum.

Negative controls

They were carried out by omitting the polyclonal antibodies.

Table 2 Patient follow up

Postoperative time (months)	Available for follow up	Dead	Lost to follow up
12	99	0	1
24	91	4	5
36	81	6	13
48	75	7	18
60	63	7	30

Statistical analysis

We studied graft survival with the Kaplan-Meier method and compared the data with the log rank test.

RESULTS

Crossmatch assays

Crossmatch assays with the anti-IgG antibody gave constant strong staining of the recipient corneal epithelium, stromal lamellae, keratocytes, and endothelium with no relevant variations between recipients. This assay was then irrelevant for predicting rejection.

Crossmatch assays with the anti-IgM antibody gave constant strong staining of the recipient corneal epithelium and endothelium, but variable staining of the recipient corneal stromal lamellae and keratocytes. Donor controls showed no corneal staining. Negative controls showed no staining. It appeared that stromal lamellae staining and keratocyte staining could be used as results of IgM crossmatch assays by grading the staining from 0 to +++ (fig 1). A stromal lamellae staining of ++ or more was found in 14 cases. A keratocyte staining of ++ or more was found in 28 cases. In these cases of positive IgM crossmatch, dilution of recipient serum up to 1/800 did not modify the results of the IgM crossmatch. Positive recipient sera were processed with negative donor corneoscleral rim which gave no staining, indicating that the antistromal IgM found in some recipient sera were specific for donor corneal antigens. There was a significant association between stromal lamellae staining and keratocyte staining ($p = 0.0004$, Fisher's exact test). Out of 14 crossmatch assays with a stromal lamellae staining of ++ or more, 10 (71%) had a keratocyte staining of ++ or more. There was no significant association between donor recipient ABO compatibility and keratocyte staining ($p = 0.25$, Fisher's exact test). There was no significant association between donor recipient HY compatibility and keratocyte staining ($p = 0.49$, Fisher's exact test).

Graft outcome

The 5 year graft survival and rejection-free graft survival (fig 2) estimates were, respectively, 71.2% and 83.3%. Graft failure occurred in 26 out of the 100 eyes. Allograft rejection was the leading cause of failure as it occurred in 15 eyes (58%). No primary graft failure occurred. No significant influences of IgM crossmatch results on graft survival were found (stromal lamellae staining, $p = 0.37$; keratocyte staining, $p = 0.91$). No significant influences of IgM crossmatch results on rejection-free graft survival were found (stromal lamellae staining, $p = 0.15$, fig 3; keratocyte staining, $p = 0.99$, fig 4). Different graft sizes did not modify these results. No significant influence of IgM crossmatch results on rejection-free graft survival was found in recipients with graft sizes larger than 8 mm (stromal lamellae staining, $p = 0.21$; keratocyte staining, $p = 0.87$) nor were they found in recipients with graft size 8 mm and less (stromal lamellae staining, $p = 0.56$; keratocyte staining, $p = 0.92$).

DISCUSSION

The tissue crossmatch technique that was used in this study allowed us to show the presence of donor specific antistromal cornea IgM in some recipients. The results of the IgG crossmatch assays can be explained by the presence of IgG in normal cornea,¹⁰⁻¹² which results in constant staining of the recipient cornea in donor controls but also in cross-matching. The recipient anti-donor corneal stroma IgM were directed against donor keratocytes or stromal lamellae. They were specific for donor corneal antigens showing they were alloantibodies. However, as no crossmatch between recipient serum and recipient cornea was performed, the presence of autoantibodies directed against corneal antigens could not be

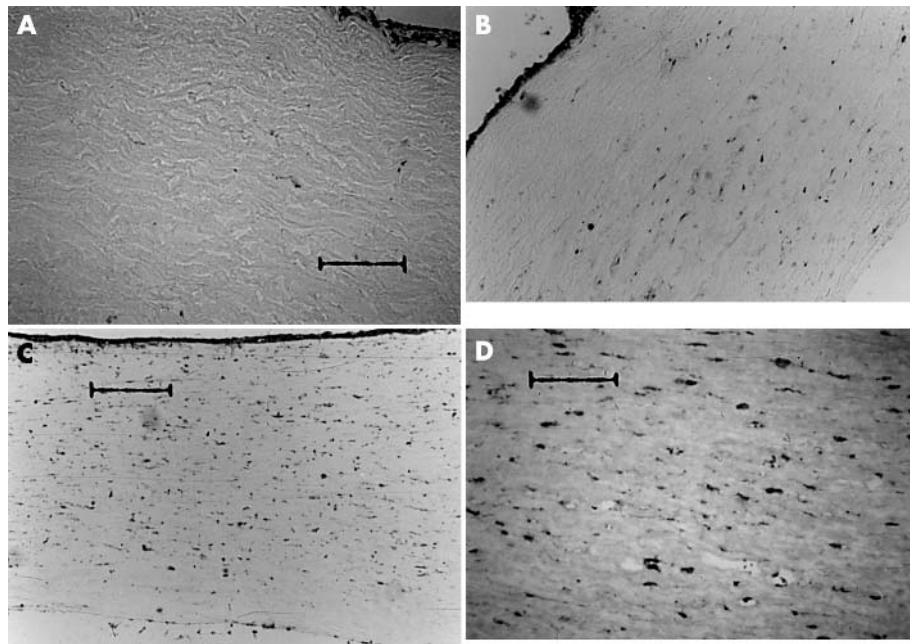


Figure 1 Grading of IgM crossmatch from 0 to +++ for stromal lamellae staining. (A) 0; (B) and (C) +; (D) +++; and keratocyte staining. (A) +; (B) ++; (C) and (D) +++.

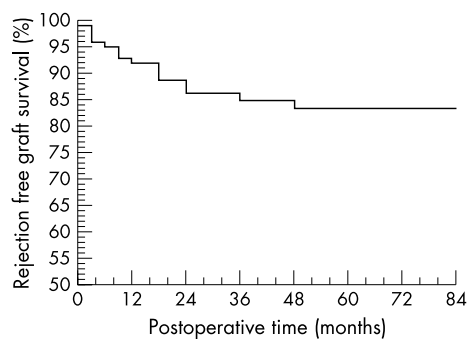


Figure 2 Overall rejection-free graft survival.

excluded. The specificity of the donor specific anti-stromal cornea IgM found in the present study cannot be determined. No significant association between ABO incompatibility or HY incompatibility and the presence of these IgM could be

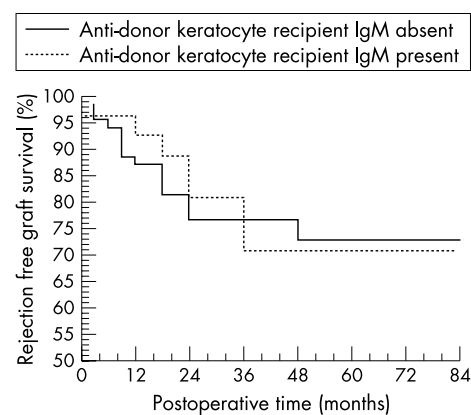


Figure 4 Rejection-free graft survival according to keratocyte staining ($p=0.99$).

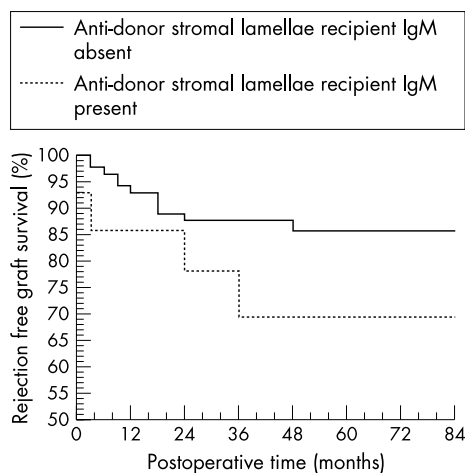


Figure 3 Rejection-free graft survival according to stromal lamellae staining ($p=0.15$).

found. These antibodies may correspond to various IgM directed against different antigens and further studies are needed to characterise their specificity.

No influence of these alloantibodies on graft survival or graft rejection could be demonstrated in this study despite the long term follow up of recipients. A previous study¹³ showed the presence of anti-bovine corneal extract IgM (1/30) or IgG (5/30) before penetrating keratoplasty in some recipients with no association with subsequent rejection of corneal transplant. These results are in contrast with those reported for kidney transplantation. Crossmatching by immunofluorescence using sections of donor skin was used to predict early kidney transplant rejection.¹⁴⁻¹⁶ It could significantly predict early rejection when patients with antibodies staining autologous skin were excluded. This difference between results obtained with kidney transplantation and those obtained with corneal transplantation may be explained by differences in the role of alloantibodies in rejection mechanism. In fact, cell mediated immunity was shown to be the main mechanism of corneal allograft rejection.¹⁷ Conversely, the antibody mediated immune reaction is strongly involved in early organ transplantation

rejection. However, antibody mediated immunity could also play a part in corneal allograft rejection. Alloantibody was shown to produce complement dependent injury to corneal allografts in mice.¹⁸ A rise in the tear IgG level was observed during a bilateral simultaneous graft rejection.¹⁹ Antibody dependent cell mediated cytotoxicity was demonstrated during a corneal graft rejection induced by corneal transplantation in the fellow eye.²⁰

These results suggest that antibody mediated immunity is unimportant in the mechanism of human corneal allograft rejection.

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